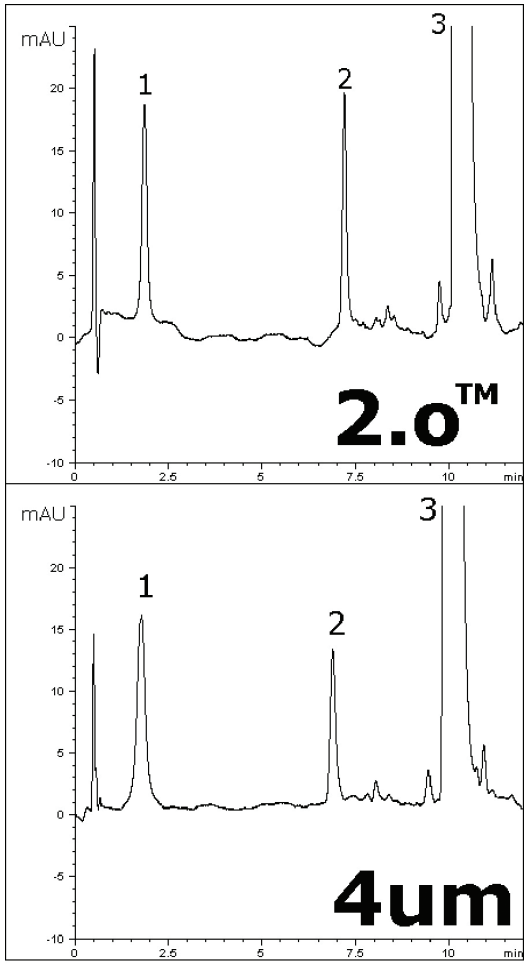
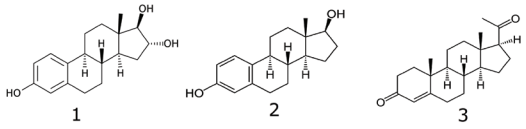
Bi-Est® Method Transfer

4µm to Near-UHPLC – Separation of Hormones





Sample: The contents of a capsule containing 0.124 mg estradiol, 1.001 mg estriol, and 50 mg progesterone were added to a 25 mL volumetric flask. The flask was diluted to mark with 5% solvent A / 95% solvent B and sonicated 10 min. Then a portion was filtered with a 0.45µm nylon syringe filter (MicroSolv Tech. Corp.). Peak identities were confirmed by individual standards.

Method Conditions

Column: **Cogent Bidentate C18 2.0̄™**, 2.2µm,1 20Å

Catalog No.: 40218-05P-2

Dimensions: 2.1 x 50 mm

Solvents: A: 90% DI water / 10% acetonitrile / 0.1% formic acid (v/v)
B: Acetonitrile / 0.1% formic acid (v/v)

Gradient:

time (min.)	%B
0	20
2	20
11	80
12	20

Post time: 5 min

Injection vol.: 2µL

Flow rate: 0.3mL/min

Detection: UV 210 nm

Peaks: 1. Estriol
2. Estradiol
3. Progesterone

Discussion

Separation of three components of a hormone replacement formulation is demonstrated in this application note using the Cogent Bidentate C18 2.0̄ column. The two figures demonstrate how comparable retention can be obtained for both the near-UHPLC column as well as the standard 4 µm column, allowing for easy method transfer. As an example of the advantages for the 2.0̄ column, the calculated efficiency for peak 2 was 201,360 plates/m for the 4µm column and 383,800 for the 2.0̄.

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